b) optionally a non-ionizable ligand covalently attached thereto,

wherein about 50 percent or more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or low ionic strength.

Cancel Claim 6 without prejudice.

R E M A R K S

It is respectfully requested that this application be reconsidered in view of the above amendments and the following remarks and that all of the claims remaining in this application be allowed.

Interview

At the outset, the undersigned wishes to thank Examiner Weber for the courtesies extended to himself and joint inventor Landon Steele during the interview conducted for this application on February 1, 1996. The substance of this interview is accurately reflected in the Interview Summary provided by the Examiner as well as in the comments set forth below.

Amendments

Applicants have amended the specification to correct several obvious errors found at page 2, line 9; at page 5, line 18; at page 10, line 14; at page 17, line 25; and at page 40, line 7.

In compliance with 37 C.F.R. §1.823(a), Applicants have also amended the specification at pages 42 and 52 to include relevant sequence information. In this regard, during a review of this application, the undersigned noted that the peptide sequence recited at page 42 requires a sequence listing and, accordingly, such a sequence listing is provided herewith.

Consistent with the discussions held during the interview, Applicants have amended Claims 1 and 16 to recite that the pH of binding the target protein or peptide to the resin is 5 or more. Support for this amendment is found in Applicants' specification at, for example, page 29, lines 24-28, as well as Example X(A) (binding at pH 9.1). Presentation of these amendments is without prejudice to Applicants' pursuing the canceled subject matter in a continuation application.

In view of the amendments to Claim 1, Applicants have canceled Claim 6 without prejudice to their pursuing this canceled subject matter in a continuation application.

Claims 1 and 16 have been further amended to recite that the pH of binding the target protein or peptide to the resin is different from the pH for desorbing this protein or peptide from the resin. Support for this amendment is found in Applicants' specification at, for example, page 28, lines 22 et seq., as well as in Example X(A) (which illustrates binding at pH 9.1 and desorption at pH 5.2) and Example X(B) (which illustrates binding at pH 7.7 and desorption at pH 5.2).

Entry of these amendments is earnestly solicited.

In view of the above, Claims 1-5 and 7-23 are now in this application.

Sequence Listing Statement

The attached computer-readable copy of the "Sequence Listing," and corresponding paper print-out are submitted to comply with the requirements of 37 C.F.R. §§1.821-1.825. Applicants attest that the information in the "Sequence Listing" is identical to that which is in computer-readable form, as required by 37 C.F.R. §1.821(f). This submission introduces no new matter since the enclosed sequences are identical to the sequences which were submitted in the original patent application.

Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 1-23 stand rejected under 35 U.S.C. §112, second paragraph, because these claims are allegedly confusing in that the resins are claimed as both uncharged and charged. For the following reasons, this reection is traversed.

Initially, Applicants note that whether the resins are uncharged or charged is a function of pH and that Claims 1-23 merely recite that the resins are uncharged at the pH target protein or peptide binds to the resin. Claims 1-23 further state that at the pH of desorption the resins are charged and these claims have been amended to state that the pH of desorption is *different* from that of protein or peptide binding. Such a characterization is necessary to distinguish over, for example, hydrophobic interaction chromatography (HIC) resins which employ a resin incapable of having a charge at *both* the pH of binding and the pH of desorption. In fact, such HIC resins can employ the same pH for both binding and desorption.

Accordingly, Applicants submit that the amended claims are not indefinite because they correctly characterize the resins as being uncharged when protein or peptide is bound thereto and charged when the protein or peptide is desorbed therefrom. Moreover, the claimed invention recites that the pH employed to bind the protein/peptide to the resin is different from the pH employed to desorb the protein or peptide from the resin.

Withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. §103

Claims 1-23 stand finally rejected under 35 U.S.C. §103 over Sasaki, et al., *J. Biochem.*, 86:1537-1548 (1979) ("Sasaki '79") or Sasaki, et al., *J. Biochem.*, 91:1551-1561 (1982) ("Sasaki '82") in view of Kasche, et al., *J. Chromatogr.*, 510:149-154 (1990) ("Kasche"), Teichberg, *J. Chromatogr.*, 510:49-57 (1990) ("Teichberg") and Jost,

et al., *Biochem. Biophys. Acta*, 362:75-82 (1974) ("Jost") and, if necessary, in view of Degen, et al., U.S. Patent No. 4,693,985 ("Degen"). For the following reasons, this rejection is traversed.

Applicants' invention provides for a complex of a resin-target protein or peptide. The resin in this claimed complex is characterized, in part, as being electrostatically uncharged where the target protein or peptide is bound to the resin at a pH of 5 or more and is further characterized as being electrostatically charged at the pH of desorption. Still further, Applicants' claimed invention recites that the pH where the protein or peptide is bound to the resin is different than the pH where the protein or peptide is desorbed from the resin.

Among other factors, Applicants' presently claimed invention is based on the discovery that resin-protein/peptide complexes wherein the resin is electrostatically uncharged at the pH where the protein or peptide is bound to the resin provides, in part, for an efficient binding of the protein or peptide to the resin for example from an aqueous media having either low or high ionic strength. Moreover, a pH of 5 or more when the protein or peptide is bound to the resin avoids the use of strong acidic conditions which can denature some proteins/peptides. See, for example, page 29, lines 24 et seq., of Applicants' specification.

Applicants maintain that this §103 rejection of Claims 1-23 over the cited references is in error because these references, either alone or in combination, simply do not teach or suggest Applicants' now claimed invention.

Specifically and as discussed during the interview, the Kasche reference shows binding of proteins to a resin which, under the conditions employed by Kasche, contain significant positive charge.

As to the Teichberg reference, it is directed to affinity-repulsion chromatography whereas Applicants' methods are not directed to affinity chromatography. Second and more to the point, Teichberg recites at page 54 et seq. that the matrices employed therein are charged when the protein is bound to the resin. Such a requirement is contrary to Applicants' claimed invention.

The Jost reference similarly discloses the presence of charged groups (i.e., positively charged groups) when the protein is bound to the resin. Additionally, Jost compares such charged resins to uncharged hydrophobic ligands which bind proteins such as BSA but fails to disclose that the resins should be capable of electrostatic charge to effect desorption of the protein or peptide from the resin. In fact, it is unclear from Jost's Table 1, whether the uncharged resins depicted in this table have a free amino group bound to the agarose or whether this amino group is part of an amide, carbamate, etc. functionality. These latter functionalities are not believed to be capable of electrostatic charge at pHs of greater than 5.

As also noted during the interview, the Degen reference is not germane to Applicants' claimed invention but rather is directed to a method for immobilizing biologically active substances or materials as acceptor molecules on active membranes. In Degen, the biologically active membrane contains an acceptor molecule which is capable of forming a biospecific complex with a second biologically active material.

In summary, as Applicants maintained during the interview, none of the secondary references cited in the §103 rejection is, in fact, germane to the now claimed complexes.

As to the two cited Sasaki references and notwithstanding statements by Sasaki to the contrary, Applicants argued during the interview that the data in each of these Sasaki references was clearly in error to one skilled in the art because the skilled artisan

would recognize that pKa's associated with carboxyl groups would necessitate that Sasaki's resins would carry considerable charge at the pHs Sasaki exemplifies for binding (i.e., pH's 4-4.5). This argument is repeated herein and Applicants maintain that the data found in the declaration of Nathaniel Todd Becker under 37 C.F.R. §1.132 for the parent application (U.S. Serial No. 08/268,178) substantiates this point. Namely, this data shows titration curves for Amberlite CG-50 resins wherein the fully protonated form of this resin is achieved at a pH significantly below pH 4.

In any event, Applicants also argued during the interview that the examples of provided in both Sasaki references were limited only to Amberlite resins which would bind the protein or resin at pHs of 4.5 or less. Contrarily, the now claimed invention has been amended to recite the resin bound with the protein/peptide is at a pH of 5 or more. In view of the narrow exemplification provided by the Sasaki references coupled with the clearly erroneous allegations of the pH at which Amberlite CG-50 resins were electrostatically neutral, Applicants submit that these references fail, by themselves (either alone or in combination), to provide any motivation to the skilled artisan to arrive at the now claimed invention.

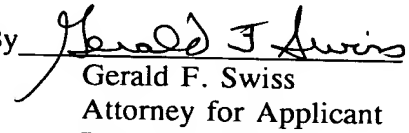
Applicants further submit that the secondary references cited in the §103 rejection fail to cure the defects of the Sasaki references because each of these references fail to disclose any reason to form a resin/protein-peptide complex wherein the resin is electrostatically uncharged at the pH of binding the protein or peptide but which will be electrostatically charged at the pH of desorption of the protein or peptide.

Applicants, therefore, maintain that this rejection is in error. Withdrawal of this rejection is earnestly solicited.

In view of the above, withdrawal of all of the objections and rejections and allowance of all of the claims remaining in this application is earnestly solicited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By 

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